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## How the epigenome contributes to the development of psychiatric disorders

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### Abstract

*Epigenetics* commonly refers to the developmental process by which cellular traits are established and inherited without a change in DNA sequence. These mechanisms of cellular memory also orchestrate gene expression in the adult brain and recent evidence suggests that the “epigenome” represents a critical interface between environmental signals, activation, repression and maintenance of genomic responses, and persistent behavior. We here review the current state of knowledge regarding the contribution of the epigenome toward the development of psychiatric disorders.

### Keywords

epigenetic; psychiatric disorders; development; histone; methylation

### Introduction

Developmental psychobiologists interested in the question of nature vs. nurture seek to understand how the genome is connected to environmental signals, and how this interaction subsequently shapes brain development and behavioral function across the lifespan. Epigenetic mechanisms provide an attractive concept for such experience-dependent mediators of environmental signals. In contrast to genetic information, the epigenome is dynamic and in principle can change in response to a variety of experience-dependent factors, including exposure to drugs of abuse, environmental toxins, nutritional factors and social contact. Within the promoter region of genes in all cell types, post-translational modification of histone proteins and covalent modification of DNA regulate the accessibility of transcription factors and directly influence gene expression. The apparatus that controls access to DNA by way of chromatin therefore represents an important interface between environmental signals, activation and repression of genomic responses, and in extreme cases, maladaptive behaviors. A number of preclinical studies have demonstrated that epigenetic regulatory mechanisms contribute to behavioral phenotypes in models of schizophrenia and depression, drug addiction, and fear-related anxiety disorders (Tremolizzo et al., 2002; Levenson et al., 2004; Yeh et al., 2004; Kumar et al., 2005; Tsankova et al.,

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2006; Bredy et al., 2007; Renthal et al., 2007; Bredy & Barad, 2008; Pandey et al., 2008). The field of epigenetics has evolved beyond an abstract concept of gene-environment interactions into a sophisticated perspective on the basic mechanisms of gene regulation, which occur at the interface between a dynamic environment and a static genome. In this article, the interaction between the genome, the epigenome and the environment, specifically as it relates to the long-term regulation of transcriptional programs associated with psychiatric disorders, will be discussed.

## Epigenetic regulation of gene expression

Epigenetic events have been defined as structural adaptation of chromosomal regions that register, signal and perpetuate altered activity states (Bird, 2007). More simply, epigenetics refers to genome information not encoded in the DNA sequence, with the best-understood consequence of epigenetic marks being the control of gene expression (Calliman and Feinberg, 2006; Feinberg, 2007). In general, epigenetic modifications function to establish and maintain different gene expression programs in specific cell types, leading to phenotypically different tissues despite each cell sharing the same genetic information (Ng and Gurdon, 2008). Epigenetic mechanisms principally include several main ways of regulating gene expression, with the sum total of all epigenetic patterns in a cell being called the “epigenome”. Epigenetic processes are tightly linked to chromatin, as this nucleoprotein complex functions as their principal template. The basic building block of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around an octamer of four histone proteins (H2A, H2B, H3 and H4) (Luger et al., 1997). The best understood epigenetic mechanism is covalent modification of the DNA itself. In eukaryotes ranging from plants to humans, DNA methylation is found exclusively at cytosine residues, primarily in the context of CpG dinucleotides (Suzuki and Bird, 2008). In the promoter region of genes, methylation of cytosines, located in so called CpG islands, is sensed by proteins that turn gene expression on or off, largely through the recruitment of histone modifying complexes (Miranda and Jones, 2007). The second and more complex kind of alteration affecting gene expression is chemical modification (acetylation, methylation, phosphorylation and many more) of the histone proteins around which chromosomal DNA is wrapped (Kouzarides, 2007). The incorporation of histone variants in place of canonical histones into nucleosomes, ATP-dependent chromatin remodeling of nucleosomes, and non-coding RNA mediated gene regulation are additional processes involved in creating the highly diverse and specialized epigenome landscape.

## Epigenetic modifications at the interface between transient gene regulation and stable changes in gene expression

Conceptually, the timing and mechanisms by which a given pattern of chromatin modification changes from transient effects on gene regulation to more persistent epigenetic programming of gene expression is currently being debated (Ptashne, 2007; Madhani et al., 2008). For the purpose of this review, we include both as each illustrates a distinct aspect of importance for chromatin-based mechanisms in the initiation, development and maintenance of the psychiatric disease phenotype. Recently, an operational model for epigenetic control of gene expression has been proposed, which involves multiple mechanistic steps leading to the establishment of a stable inherited epigenetic state (Berger et al., 2009). First, an environmental cue or changes in the niche of a cell or organism trigger intracellular signaling pathways that, in turn, directly interact with the chromatin structure. This initial signal will be transient and result in a second step in the initiation of an epigenetic event, which generally is the establishment of a local chromatin context at a precise chromosomal location. In the context discussed here, this initiation event is tightly linked to the sequence-

specific DNA binding by transcription factors, or non-coding RNAs. Lastly, once established, the altered chromatin environment needs to be stably maintained, so that the epigenetic signal can function through the cell cycle, or persist in terminally differentiated cell types. This last step involves many different pathways such as DNA methylation, histone modifications, histone variants, nucleosome positioning and others (Berger et al., 2009). Importantly, the maintenance state cannot occur in the absence of the initiation and establishment phase because sequence-specific DNA recognition events and transcriptional networks control the majority of epigenetic changes. However, illustrating the delicate circuitry and tight interdependence of these important regulatory processes, these same transcriptional networks that control epigenetic patterns are acting upon DNA packaged by histones into nucleosomes, hence on an established epigenetic landscape (Panning and Taatjes, 2008).

In this model, an important distinction between transient and persistent takes place at the conclusion of the initiation event and the beginning of the establishment stage. If the latter involves only labile chromatin modifications, it will only result in transient gene induction. Functionally, post-translational modifications of histone proteins are squarely at the interface between the very dynamic processes of gene induction, and maintenance of stable epigenetic signals. The majority of histone modifications correlate with activating or repressive function, dependent on which particular residue in the histone is modified and the exact chemical nature of the modification (Kouzarides, 2007). While most genes are associated with one of a few patterns of chromatin modification, some histone modifications are highly dynamic and rapidly change in response to changes that turn genes on or off (Bhaumik et al, 2007). Hence, these particular modifications would not suffice to establish permanent marks.

## Histone modification

Histone modifications have recently attracted much attention because of their central role in gene regulation. The N-terminal tails of the core histones, H3 and H4, can be modified by acetylation, methylation, phosphorylation, ubiquitination, and sumoylation (Jenuwein & Allis, 2001). Histone modifications representing an active histone code are acetylation of histone tails and di- or tri-methylation of histone H3 lysine 4 (H3K4me<sub>2/3</sub>). In contrast, histone modifications non-permissive for gene transcription (inactive code) are deacetylation of histone tails and di- or tri-methylation of histone H3 lysine 9 (H3K9me<sub>2/3</sub>). Histone H3 lysine 27 (H3K27me<sub>3</sub>), which is catalyzed by the polycomb group (PcG) protein complex, also represents an inactive histone code and the coexistence of H3K4me<sub>2/3</sub> and H3K27me<sub>3</sub> indicates a repressed, but “poised”, chromatin state. Enzymes that catalyze histone acetylation/deacetylation are histone acetyltransferases (HATs, i.e. CBP/p300) and histone deacetylases (HDACs, i.e. HDAC5). The enzymes that regulate histone methylation are histone methyltransferases (HTMs, such as MLL, G9a, Suv39H1, and EZH2) and histone demethylases (HDMs, such as JARID1d and Utx) (Lachner & Jenuwein, 2002; Lachner et al., 2003). Interestingly, while H3K4me<sub>3</sub> renders competence for transcriptional activation H3K9me<sub>3</sub> is tightly associated with Dnmt3a and DNA methylation (Ohm et al., 2007).

## DNA methylation

The first epigenetic modification to be described in the mammalian genome, DNA methylation specifically occurs on cytosines located at the 5<sup>th</sup> C position of the pyridine ring, within CpG dinucleotides on double stranded DNA (Ng and Bird, 1999; Tucker, 2001). *De novo* DNA methylation is catalyzed by two *de novo* DNA methyltransferases, Dnmt3a and Dnmt3b (Ng & Bird, 1999; Tucker, 2001). DNA methylation often leads to gene silencing, and is critically involved in early embryogenesis, maintenance of genome

stability, X-chromosome inactivation, genomic imprinting, and tumorigenesis (Akbarian et al., 2005; Chen et al., 2003). DNA methylation at transcription factor binding sites often attenuates the association of the transcription factor to the DNA, which can cause repression of gene expression (or even increased gene expression when the methylated site is that of a transcriptional repressor). The primary mechanism by which DNA methylation inhibits gene expression is mediated through methyl-CpG binding proteins, such as MeCP2, and MBDs, which recruit histone modification enzymes to the methylated DNA and modulate chromatin structure (Chen & Li, 2004).

## Epigenetic mechanisms in schizophrenia

The role of the epigenome in the aetiology of schizophrenia has been hypothesized for well over thirty years (Osmond and Smythies, 1952). In the early 1960's, it was discovered that chronic administration of L-methionine, a methyl donor and S-adenosyl-methionine precursor noted for its anti-depressant effects, exacerbated symptoms in schizophrenics, which provided the first support for the "transmethylation hypothesis" of psychosis (Pollin et al., 1961). When the group of Costa, Grayson and Guidotti at the University of Illinois (UIC) discovered that the promoter region for reelin, a synaptic plasticity-related gene whose expression is reduced by ~50% in GABAergic cortical interneurons of patients with schizophrenia, was hypermethylated in those those patients, the investigation of the epigenetic hypothesis of schizophrenia and its molecular underpinnings re-emerged (Guidotti et al, 2000; Grayson et al., 2005). The association between reelin promoter hypermethylation, DNMT1 activity, and reelin mRNA expression in schizophrenia has been confirmed by postmortem analysis in three separate laboratories (Abdolmaleky et al., 2005; Ruzicka et al., 2007; Tamura et al., 2007), however, Tochigi et al (2008) failed to replicate the finding.

In order to gain further insight into the epigenetic mechanisms of gene regulation in schizophrenia, the UIC group developed a mouse model using protracted L-methionine treatment in both wild-type (WT) and heterozygous *reeler* mice (HRM) (Tremolizzo et al., 2002). HRM show decreased synaptic plasticity and down regulation of reelin and GAD67 gene expression, similar to that reported in schizophrenia (Akbarian et al, 1995; Guidotti et al., 2000). Chronic L-methionine treatment causes a marked reduction in reelin and GAD67 mRNA expression in both WT and HRM, and this effect is associated with increased DNA promoter methylation and an impaired type of sensorimotor gating called prepulse inhibition (PPI), which is also impaired in schizophrenia (Tremolizzo et al., 2002). Interestingly, by increasing histone acetylation levels using the non-specific HDAC inhibitor sodium valproate (VPA), the L-methionine-induced impairment in PPI, DNA promoter methylation and its correlated down regulation of reelin and GAD67 gene expression, was normalized (Tremolizzo et al., 2002). Dong et al. (2005) expanded on these findings by demonstrating that protracted L-methionine treatment leads to increased recruitment of the methyl-CpG binding proteins MeCP2 and MBD2 to the promoters of reelin and GAD67. Importantly, these effects are restricted to GABAergic neurons and are reversed by concomitant treatment with VPA, possibly by disrupting a multi-protein repressor complex or by inducing putative DNA demethylase activity. Indeed, Dong et al (2008) recently demonstrated that two antipsychotic drugs, clozapine and sulpiride, induce rapid demethylation of the reelin and GAD67 gene promoters and these effects are potentiated by VPA. Further evidence for a role of active DNA methylation in the functional repression of reelin and GAD67 comes from *in vitro* studies, where activation of reelin and GAD67 leads to a reduction in DNMT1 protein levels, a dissociation of DNMT1 and MeCP2 from their promoters, and DNMT inhibitors further enhance this relationship (Noh et al., 2005; Kundakovic et al., 2007; 2009). Nicotine down regulates DNMT1 mRNA and protein expression, decreases methylation of the GAD67 promoter, and prevents the effects L-

methionine exposure (Satta et al., 2008). These are interesting findings given that smoking is a common method of self-medication in schizophrenia (Leonard et al., 2007), and while nicotine is not known to directly inhibit DNMTs, it may find indirect use in reversing epigenetic modifications of genes whose expression is impaired in schizophrenia.

More recently, the relationship between DNA methylation and schizophrenia has been examined using an epigenome-wide profiling approach. Mill et al (2008) assayed tissue derived from schizophrenia, bipolar disorder and control subjects using CpG-island microarrays to examine ~7800 individual CpG rich regions followed by pyrosequencing for validation. They identify epigenetic modifications related to glutamatergic and GABAergic neurotransmission, along with neuronal development and metabolism. Interestingly, they also describe significant sex differences in DNA methylation across several loci associated with psychosis. Notwithstanding certain confounding variables such as tissue heterogeneity and the dynamic temporal nature of DNA methylation (Miller and Sweatt, 2007; Connor and Akbarian, 2008) this study advances our appreciation for the role of DNA methylation in schizophrenia.

DNA methylation isn't the only epigenetic mechanism that is active during early development of schizophrenia. As described earlier, many histone modifications are intimately associated with either dynamic gene induction or persistent programming of gene expression and may therefore play a more direct role in aberrant gene expression profiles observed in schizophrenia. There is a decrease in H3K4me3 and an increase in H3K27me3 around the promoter for the GABA-related gene, GAD1/GAD67, which is accompanied by decreased GAD mRNA expression (Huang et al., 2007a). These effects are mediated in part by the recruitment of the H3K4-specific methyltransferase mixed-lineage leukemia (Mll1) to the GAD67 promoter and, interestingly, this recruitment along with its permissive influence on gene expression is increased by antipsychotic drug treatment (Huang et al., 2007b).

## Epigenetic mechanisms in drug addiction

In recent years, Nestler and colleagues have led the field in describing how the epigenome contributes to neural and behavioral adaptation associated with drugs of abuse (Renthal and Nestler, 2008). In their groundbreaking study, Kumar et al., (2005) demonstrate how exposure to behaviorally relevant doses of cocaine lead to histone modifications and subsequent expression of several genes known to be critical for development of drug-seeking behavior. Acute cocaine exposure induces to a time-dependent increase in H4 acetylation and phospho (Ser10)-acetylated H3K14 around the promoter of the immediate early gene (IEG) *c-Fos* with a peak at 30–90 min and return to baseline 180 min post cocaine administration. After chronic cocaine exposure, while *c-Fos* remains unaffected, there is a significant increase in H3K9 and K14 acetylation around the promoter of the IEG *FosB*. This effect occurs after both passive cocaine administration and under conditions of chronic self-administration. Remarkably, histone modifications around both the *c-Fos* and *FosB* promoters map on to the time-dependent nature of IEG expression in response to acute and chronic cocaine exposure, which therefore suggests that distinct patterns of chromatin remodeling are associated with specific genes that exhibit either acute or long-lasting expression profiles. Interestingly, *FosB* codes for the transcription factor  $\Delta$ FosB, which is implicated in the transition from acute recreational drug use to chronic addiction (McCLung and Nestler, 2003).  $\Delta$ FosB binds directly to the promoter of the *c-Fos* gene after acute exposure to amphetamine and, in contrast to its permissive effect on *Cdk5* expression, leads to repression of *c-Fos* activity (Renthal et al., 2008). Binding, desensitization, and repression of *c-Fos* gene expression are mediated epigenetically by the recruitment of HDAC1 and histone methyltransferase (KMT1A), and by increased H3K9me2 around the *c-Fos* promoter. Together, these findings suggest that epigenetic mechanisms participate in



feedback loops involving immediate early gene activity, both histone acetylation and methylation, and expression of specific genes in response to acute and chronic exposure to drugs of abuse.

Further reinforcing the idea of gene specificity in mechanisms of chronic cocaine seeking, Kumar et al. (2005) demonstrate that the promoters of BDNF and Cdk5, which are induced only after chronic exposure to cocaine, show increased H3K9 and K14 acetylation. Intriguingly, H3K9 and K14 acetylation around the BDNF promoter persists for up to a week after drug exposure ends, suggesting that active epigenetic mechanisms regulate long-term expression of BDNF, and may thus explain time-dependent increases in BDNF and cocaine-induced craving after withdrawal (Grimm et al., 2003). Further, the effect of cocaine on histone modifications in the striatum can be enhanced by concurrent systemic administration of HDAC inhibitors (NaBt or trichostatin A (TSA)) and these effects parallel increased in behavioral sensitivity to cocaine; effects which can be blocked by overexpression of HDAC4 in nucleus accumbens (Kumar et al., 2005). These findings parallel earlier work showing a critical role for histone acetyltransferase (HAT) activity in cocaine sensitization (Levine et al., 2005).

As a follow up, Renthal et al. (2007) demonstrate that intra-nucleus accumbens (NA) infusion of the class I-II HDAC inhibitor suberoylanilide (SAHA) enhances cocaine-induced conditioned place preference (CPP). Then, in an effort to establish a specific role of epigenetic regulatory mechanisms in cocaine addiction, Renthal et al. (2007) show that the class II histone deacetylase, HDAC5, is highly expressed in NA, that acute exposure to cocaine activates HDAC5, and that overexpression of HDAC5 blocks the rewarding effects of cocaine. Further, after chronic exposure to cocaine, HDAC5 knockout (KO) mice exhibit exaggerated cocaine-seeking behavior and enhanced avoidance behavior after chronic exposure to emotional stress. Impressively, overexpression of HDAC5 within the NA normalizes chronic cocaine-induced drug-seeking behavior in HDAC KO mice. Renthal et al (2007) propose that HDAC5 functions as a central mediator of chronic environmental stimuli and behavioral adaptation. With regard to chronic exposure to drugs of abuse and emotional stress, they suggest that HDAC5 is critical for the transition from short-term physiological to long-term pathological behavior by mediating the epigenetic regulation of gene expression in brain regions that support both reward and aversive learning.

Dopamine receptor 1 (Drd1) mediated signaling plays an important role in the development of cocaine addiction (Self and Stein, 1992; Hummel and Unterwald, 2002; Graham et al., 2007). Schroeder et al (2008) demonstrate that Drd1 activation and/or administration of the HDAC inhibitor, NaBt, enhances cocaine-induced locomotor activity and increases cocaine-seeking behavior. Drd1-dependent H3 phosphoacetylation in striatum along with a correlated decrease in H3 acetylation and BDNF mRNA expression in ventral midbrain accompany these behavioral effects. These findings demonstrate regional specificity in the epigenetic mechanisms driving cocaine-seeking behavior. Stiponowich et al (2008) recently expanded our understanding of the important role for the Drd1 signaling cascade in mediating epigenetic and behavioral responses to drugs of abuse. Drd1 activation leads to nuclear accumulation of DARP-32, inhibition of protein-phosphatase-1 (PP1) and increases H3 phosphorylation, which together mediates behavioral responses to cocaine and natural reward. Interestingly, PP1 directly regulates histone modifications around at least two promoters, CREB and NfKappaB; two genes known to be critical for the formation of long-term memory (Graff et al., 2008). These experiments, along with the work of Kumar et al. (2005) and Renthal et al. (2007 and 2008) set the standard for future studies on the integration of epigenetic mechanisms and environmental signals, and their role in the development of drug addiction and other psychiatric disorders.

Studies on how the epigenome contributes to behavioral responses to alcohol are also emerging. The anxiolytic effect of acute alcohol exposure is associated with increased neuropeptide Y (NPY) mRNA expression, decreased activity of class I–II HDACs and global increases in H3 and H4 acetylation within the central and medial nucleus of the amygdala (Pandey et al, 2008). In contrast, after chronic exposure to alcohol, withdrawal-induced anxiety-related behavior is associated with decreased global histone acetylation, HDAC activity and expression of NPY. Importantly, these effects can be reversed by systemic administration of the HDAC inhibitor, TSA (Pandey et al., 2008). These important findings extend the role of the epigenome in mediating behavioral responses to drugs of abuse to include the amygdala, a brain region strongly implicated in addiction-related behavior. They are also reminiscent of the Kumar et al (2005) study where acute and chronic cocaine exposure involves very different epigenetic mechanisms. However, the correlative nature of NPY mRNA expression, global histone modifications and HDAC activity does not establish a causal relationship between epigenetic mechanisms regulating gene expression and the development of alcohol seeking-behavior. It would be of interest to determine the precise epigenetic profile of the promoter for NPY in an alcohol self-administration paradigm, and to determine if specific epigenetic mechanisms targeting NPY could functionally reverse alcohol withdrawal-induced anxiety and craving.

In an interesting experiment by Pascual et al. (2009), the question of how the epigenome contributes to vulnerability or predisposition for alcohol abuse is raised. Chronic, intermittent (binge-like) alcohol exposure to juvenile rats increases subsequent alcohol seeking behavior in adulthood and is associated with increased baseline DA efflux and H3K9 and H4K12 acetylation in frontal cortex and nucleus accumbens (Pascual et al., 2008). These findings will be strengthened by future studies detailing the functional relevance of a dynamic epigenome after exposure to alcohol during critical periods of development, and the specific role it plays in sensitizing or priming gene expression in response to subsequent alcohol seeking behavior.

As of yet, few studies have examined the role of DNA methylation in addiction. Cassel et al (2006) found increased in MeCP2 and MBD1 and concomitant HDAC2 protein expression in the caudate-putamen after 10 days of cocaine exposure, and acute methamphetamine exposure decreases DNMT1 and DNMT2 mRNA expression in the hippocampus of rats (Numahci et al, 2004). In peripheral blood cell samples derived from alcohol-dependent human subjects, there is significant hypermethylation within the promoter of the vasopressin, dopamine transporter (DAT) and alpha-synuclein genes; all of which are known to play a role in addiction-related behavior (Bonsch et al., 2005; Hillemecher et al, 2008a, 2008b). While the true functional relevance of these effects remains to be determined, we catch a glimpse of it in subjects experiencing the lowest levels of craving after withdrawal who, interestingly, show the highest DAT promoter methylation levels. These findings are a positive step toward establishing a role for DNA methylation in drug addiction; however, a major limitation in the interpretation of studies such as those described, is the use of peripheral blood to measure epigenetic modifications. Given the important role of epigenetic mechanisms in establishing cellular differentiation and gene expression patterns leading to the more than 200 specific cell types in the human body, it is not easy to imagine how one could extrapolate epigenetic effects seen in peripheral blood to gene expression in specific anatomical regions supporting addiction-related behavior. Presently, the analysis of post-mortem tissue samples (like the genome-wide study by Mill et al., 2008) appears to be the best approach to gaining insight into the role of epigenetic mechanisms in human psychiatric disorders.

## Epigenetic mechanisms in bipolar and major depressive disorder

Several groups are currently exploring how the epigenome contributes to the aetiology of depression. Tsankova et al (2004) first demonstrated histone modifications around promoters of *c-Fos*, *BDNF* and *CREB* in the hippocampus after electroconvulsive shock therapy (ECT). These findings elucidate activity-dependent epigenetic mechanisms in the adult brain and pave the way for more detailed studies on the role of the epigenome as a potential therapeutic tool in the treatment of major depressive disorder. For example, VPA, along with sodium butyrate (NaBt) when administered alone or in combination with the anti-depressant fluoxetine, improves performance in a model of behavioral despair (Semba et al., 1989; Schroeder et al., 2007).

There is a profound reduction in hippocampal *BDNF* mRNA expression after chronic social defeat (another model of depression); an effect accompanied by a long-lasting increase in H3K27me2 around two isoform specific promoters (P3 and P4) of the *BDNF* gene (Tsankova et al 2006). Interestingly, the anti-depressant imipramine reverses the effect of chronic social defeat on depression-related behavior and on *BDNF* mRNA expression by increasing histone H3 and H4 acetylation and H3K4me2 around the same promoters. Imipramine does this by overcoming the repressive influence of H3K27me2, which does not change. With respect to the findings on H3K27 and H3K4, there is some debate in the field regarding the significance of the “me2” mark. It has not yet been determined whether dimethylation signifies a transition from a repressive to permissive chromatin state, or whether it, like the well-established trimethylation (me3) mark, signifies a stable epigenetic modification. In future studies, it will be very important to distinguish between transient and stable epigenetic states, as this will have implications for understanding exactly how the epigenome contributes to long-lasting changes in gene regulation associated with psychiatric disease states.

Tsankova et al (2006) also shed light on a possible epigenetic mechanism of action for the anti-depressant effects of imipramine. They first demonstrate that the effect of imipramine on histone modifications and gene expression are present only in the chronic social defeat treatment group. Next, expression of the histone-modifying enzyme HDAC5 is down regulated in the hippocampus of socially defeated mice treated with imipramine and, amazingly, overexpression of HDAC5 by HSV viral-mediated gene transfer completely blocks the effect of imipramine on social interaction and avoidance behavior. These findings demonstrate an important role for epigenetic mechanisms in the neuroadaptive changes that occur in major depression and, perhaps more importantly, in mediating the effects of anti-depressant treatment.

Across the lifespan, differences in genome-wide methylation patterns become more apparent in mono-zygotic (MZ) twins; findings which might help to understand the high (~50%) discordance rate amongst MZ twins for depressive disorders (Fraga et al., 2006). In MZ twins discordant for psychiatric disease, including depression, there are differences in DNA methylation within the promoters of the dopamine 2 receptor (*Drd2*) and catechol-O-methyltransferase (*COMT*) genes (Petronis et al., 2003; Mill et al., 2006). However, as discussed by Mill and Petronis (2007), there are currently few studies examining the role of DNA methylation in major depression. As technological limitations are addressed and overcome, the identification of epigenetic mechanisms in major depression and other psychiatric disorders will become more accessible (Connor and Akbarian, 2008). For instance, Mill et al (2008) have begun the arduous task of genome-wide epigenetic profiling in bipolar depression. Similar to their observations in schizophrenics, the most intriguing findings are related to sex differences in DNA methylation within the cortex of subjects with



bipolar depression. These observations provide a foundation for future studies on the underlying mechanisms of sexual dimorphism in psychiatric disease.

## Epigenetic mechanisms in anxiety disorders

Insight into how the epigenome contributes to the development of anxiety disorders comes from studies on the mechanisms of fear-related learning and memory. Using a pre-clinical model of phobia, Yeh et al. (2004) demonstrate that the acquisition of cued-fear stimulates histone acetyltransferase (HAT) activity and increases the interaction between CREB-binding protein (CBP) and the promoter of NFkappaB, within the basolateral nucleus of the amygdala (BLA). These effects are enhanced by local infusion of the HDAC inhibitor trichostatin (TSA), administered 30 minutes prior to training, which parallels enhanced memory for cued-fear and also enhances LTP in the BLA. The same year, two other labs also demonstrated a critical role for CBP activity in the formation of long-term fear memory (Alcaron et al., 2004; Korzus et al., 2004). Recently, Wood, Abel and colleagues have used a transgenic approach to elucidate the role of CBP in mediating the memory enhancing effects of HDAC inhibitors (Wood et al., 2006; Olivera et al, 2007; Vescey et al., 2007).

The acquisition of contextual fear memory is associated with a global increase in H3K14 acetylation in the hippocampus, and HDAC inhibitors enhance both long-term contextual fear memory and learning-related synaptic plasticity (Levenson et al., 2004). Interestingly, Levenson et al. (2004) observe a global increase in hippocampal H4 acetylation in a model of inhibitory learning called latent inhibition, suggestive of unique epigenetic mechanisms underlying different forms of learning. Indeed, the extinction of conditioned fear shares similar molecular substrates with latent inhibition, and elicits its own unique pattern of histone modifications around individual promoters of the BDNF gene, relative to the acquisition of conditioned fear (Bredy et al., 2007). Like the acquisition of conditioned fear, fear extinction can also be enhanced by both local and systemic administration of the HDAC inhibitors (Bredy et al., 2007; Lattal et al., 2007; Bredy et al., 2008), and this effect is accompanied by increased H4 acetylation around the BDNF exon IV gene promoter within the prefrontal cortex; a brain region implicated in long-term memory for fear extinction (Bredy et al., 2007). We recently demonstrated that the effect of HDAC inhibition on fear learning and extinction is also exquisitely sensitive to the timing of HDAC inhibition relative to the behavioral paradigm. VPA, administered prior to a massed extinction protocol, leads to enhanced reconsolidation of the original fear memory. Conversely, the same dose of VPA administered prior to a spaced extinction protocol leads to enhanced long-term memory for extinction, which is rendered independent of training context (Bredy et al., 2008). These findings have important implications for targeting the epigenome by HDAC inhibition as an adjunct to cognitive behavioral therapy for fear-related anxiety disorders.

Lending further support to the idea that different learning processes are supported by unique epigenetic mechanisms, Maurice et al. (2008) show that mice lacking the HAT, p300/CBP-associated factor (PCAF), exhibit exaggerated stress reactivity, stronger acquisition of conditioned fear, and impaired reversal learning. Recent observations from our group suggest that, among other epigenetic regulatory proteins, the expression of PCAF is increased in both medial prefrontal cortex and hippocampus after extinction learning, and that local infusion of a PCAF-specific inhibitor (H3-CoA-20) into ventromedial prefrontal cortex impairs consolidation of fear extinction memory (Bredy T.W. unpublished observations). Together, these data suggest a pivotal role for PCAF as an epigenetic regulatory mechanism uniquely associated with inhibitory learning.

There is a wide body of literature describing how exposure to stress contributes to increased vulnerability for anxiety disorders (McEwen, 2008), and recent work suggests a role for the epigenome in mediating these effects. In adult rats and mice, psychological stressors such as forced-swimming, predator odor and novelty exposure, all elicit a global and transient increase in Ser10 phospho (Ser10) acetylated H3K14 within neurons of the dentate gyrus (Bilang-Bleuel et al., 2005; Chandramohan et al., 2008). These studies suggest an important role for dynamic epigenetic processes involved in stress-related learning and memory. Reul and Chandramohan (2007) propose that stress-induced behaviors are a function of the activity-dependent NMDA- and GR-signaling cascades, which converge on chromatin remodeling complexes within the dentate gyrus, thereby providing a signature for long-term emotional memories. As mentioned in previous sections, in order to provide evidence for causal relationships between epigenome and behavior future studies will need to use more sophisticated approaches to identify precise genomic targets associated with stress-related epigenetic regulation.

In a landmark series of experiments by Michael Meaney and colleagues at McGill University, a causal relationship between early life experience, epigenetic programming, and stress-related behavior as been established (Weaver et al., 2004). Offspring of mothers that receive low levels of maternal care show increased stress reactivity and anxiety-related behavior in adulthood, decreased hippocampal glucocorticoid receptor (GR) mRNA expression, and decreased activity-dependent NGFI-A transcription factor binding to the GR promoter. These effects are mediated by decreased H3K9 acetylation around, and increased DNA methylation within, the GR<sub>1-7</sub> promoter. Interestingly, in these and subsequent studies on the hippocampal transcriptome, Weaver et al. show that the effects of early life experience on epigenomic programming are very dynamic and reversible in adulthood (Weaver et al. 2005; 2006): major findings that reinforce the idea that the epigenome plays a role in mediating the effects of experience on vulnerability (and resilience) to the development of anxiety disorders. Amazingly, these findings have since been translated to humans. McGowan et al (2009) demonstrate that in suicide victims with a history of childhood abuse, the same pattern of epigenetic regulation of the GR promoter emerged. The role of epigenetic mechanisms mediating vulnerability to stress has recently been expanded. KAP1 is an essential component of a repressive chromatin complex made up of histone methyltransferase SETDB1 and histone deacetylase NuRD, which serves to silence gene transcription (Sripathy et al, 2006; Urrutia et al., 2003). Mice with a forebrain specific KAP1 deletion exhibit high levels of anxiety-related behavior and significant stress-induced impairments in attention, and in spatial learning and memory (Jakobsson et al., 2008). Genome-wide mRNA expression profiling revealed a number of genes whose expression is altered by KAP1 knockout, and which show corresponding histone modifications associated with transcriptional activation. Together, these findings suggest an important role for the epigenetic corepressor, KAP1, as a homeostatic regulator of gene expression in response to stress and support Meaney's original assertion that the epigenome makes an important contribution to experience-dependent vulnerability and resilience to stress-related anxiety disorders.

Fear-related learning and memory is also mediated, in part, by DNA methylation. In the first of a series of groundbreaking studies, Levenson et al. (2006) demonstrate that DNA methyltransferases (DNMTs) participate in hippocampal synaptic plasticity in the adult brain. The *de novo* DNMTs, DNMT3a and DNMT3b, are activated by contextual fear learning and are associated with DNA methylation within the promoter of the protein phosphatase (PP1) gene (Miller and Sweatt, 2007). Interestingly, Miller and Sweatt (2007) observe concomitant demethylation within the promoter of the reelin gene, reminiscent of activity-dependent reelin promoter demethylation after treatment with both antipsychotics and HDAC inhibitors (Dong et al., 2008). Further, contextual fear learning leads to both

increased H3 acetylation around, rapid demethylation within, the P4 promoter of the BDNF gene, and local infusion of DNMT inhibitors interferes with fear memory consolidation (Lubin et al., 2008). Together, these findings suggest a mechanistic relationship between histone modifications and DNA methylation, which is crucial for the consolidation of long-term fear memories, and which provides a potential mechanism for the development of fear-related anxiety disorder.

## Challenges and Implications

In the past decade, there has been a virtual explosion in the number of studies demonstrating the dynamic nature of the epigenome and its influence on the regulation of genes and behavior across the lifespan. The evidence strongly favors an important role for epigenetic mechanisms in mediating gene-environment interactions, and in the development of, experience-dependent, environmentally driven psychiatric disorders. As our understanding of epigenetic mechanisms in the context of brain and behavioral development increases, so too must our appreciation for higher standards in experimental design and technical approaches. For example, a number of studies examining the association of DNA methylation with behavior have relied on the methylation-specific PCR approach (MSP). While providing general information on the methylation state of a given promoter, this technique lacks single CpG resolution, which is absolutely necessary to understand how subtle epigenetic modifications within the gene impart long-term influences on gene regulation. A combined approach involving bisulphite mapping, direct sequencing, and/or mass spectrometry (MALDI-TOF) will become the gold standard for assessing the role of DNA methylation in psychiatric disease. Several other issues that need to be considered when designing epigenome experiments are; tissue heterogeneity, which can easily be overcome by cell sorting approaches prior to assay; low tissue availability, which can be overcome with techniques such as carrier chromatin immunoprecipitation assay (c-ChIP), and the use of non-specific drugs that target the epigenome. For example, certain compounds that target DNMT's (i.e. 5-aza-cytidine) are not able to inhibit DNMT activity in post-mitotic neurons, so care must be taken when considering the use of these compounds for *in vivo* studies. This is an exciting time for the fields of neuroscience and psychiatry. The technology necessary for interrogating the epigenome is rapidly evolving, and accordingly, our understanding of how epigenetic mechanisms contribute to the development of psychiatric disease will continue to be refined for years to come.

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